PALM Intranet

Application Number [ ] SEARCH

IDS Flag Clearance for Application 10043539

IDS Information

Content	Mailroom Date	Entry Number	IDS Review	Reviewer
M844	10-18-2004	32	☑	11-12-2004 12:39:53 gtrammell
M844	01-12-2006	54	☑	02-03-2006 14:28:27 jmason

UPDATE

WEST Search History

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DATE: Wednesday, April 05, 2006

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR		
☐	L1	winged near helix near protein$	41
☐	L2	forkhead or fork-head	656
☐	L3	L2.ti,ab,clm.	84
☐	L4	(sar or sara or sar-a or sarr or sar-r).ti,ab,clm.	4151
☐	L5	L4 and staph$	58
☐	L6	L5 not l1	56
☐	L7	l6 and (l1 or l2 or accessory)	11

END OF SEARCH HISTORY

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Your result set for the last L# is incomplete.
The probable cause is use of unlimited truncation. Revise your search strategy to use limited truncation.

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Search Results - Record(s) 1 through 41 of 41 returned.

☐ 1. 20060068395. 17 Sep 04. 30 Mar 06. Synthetic nucleic acid molecule compositions and methods of preparation. Wood; Keith V., et al. 435/6; 435/320.1 536/23.1 C07H21/04 20060101 C12Q1/68 20060101

☐ 2. 20060014145. 21 Mar 03. 19 Jan 06. Methods and articles for high throughput analysis of biomolecular interactions. Miller; Brian Scott. 435/6; 435/287.2 C12Q1/68 20060101 C12M1/34 20060101

☐ 3. 20050255123. 11 Jan 05. 17 Nov 05. Chimeric ebola virus envelopes and uses therefor. Wilson, James M., et al. 424/186.1; 435/235.1 435/325 435/5 435/69.3 530/350 536/23.72 C07K014/01 C12Q001/70 C07H021/04 A61K039/12 C12N007/00.

☐ 4. 20050239704. 22 Feb 05. 27 Oct 05. Compositions and methods for affecting virulence determinants in bacteria. Cheung, Ambrose L., et al. 514/12; 435/252.3 435/471 435/6 435/69.3 530/350 536/23.2 A61K038/16 C12Q001/68 C07H021/04 C12N015/74 C12N001/21 C07K014/31.

☐ 5. 20050191242. 24 Nov 04. 01 Sep 05. Foxn1 and pigmentation. Brissette, Janice, et al. 424/9.2; 424/62 A61K049/00 A61K007/42 A61K007/135.

☐ 6. 20050181369. 14 Oct 04. 18 Aug 05. Compositions and methods for diagnostics and therapeutics for hydrocephalus. Blackshear, Perry J, et al. 435/6; 435/320.1 435/325 435/69.1 530/350 536/23.5 C12Q001/68 C07H021/04 C07K014/705.

☐ 7. 20050069866. 12 May 04. 31 Mar 05. Simian adenovirus nucleic acid and amino acid sequences, vectors containing same, and methods of use. Wilson, James M., et al. 435/5; 435/235.1 435/325 435/456 435/69.3 530/350 536/23.72 C12Q001/70 C07H021/04 C07K014/075 C12N007/00 C12N015/86.

☐ 8. 20050059168. 17 Sep 03. 17 Mar 05. Conformationally flexible cationic conjugated polymers. Bazan, Guillermo C., et al. 436/518; 525/54.1 C08G063/48 C08G063/91 G01N033/543.

☐ 9. 20050019928. 17 Aug 04. 27 Jan 05. Novel compositions and methods for production of recombinant virus. Rasty, Siyamak, et al. 435/456; C12N015/86.

☐ 10. 20050014262. 08 Jun 04. 20 Jan 05. Adeno-associated virus (aav) serotype 9 sequences, vectors containing same, and uses therefor. Gao, Guangping, et al. 435/456; 435/235.1 530/350 C12N007/00 C12N015/861 C07K014/075.

☐ 11. 20050003386. 13 Feb 04. 06 Jan 05. Methods and compositions for detection and analysis of polynucleotide-binding protein interactions using light harvesting multichromophores. Bazan, Guillermo C., et al. 435/6; 525/54.2 536/25.32 C12Q001/68 C07H021/04 C08G063/48 C08G063/91.

☐ 12. 20040171807. 21 Nov 03. 02 Sep 04. Method for rapid screening of bacterial transformants and novel simian adenovirus proteins. Gao, Guangping, et al. 530/350; C07K014/075.

☐ 13. 20040166061. 23 Mar 04. 26 Aug 04. Animal model. Enerback, Sven, et al. 424/9.2; 435/6 A61K049/00 C12Q001/68.

☐ 14. 20040136963. 19 Dec 03. 15 Jul 04. Simian adenovirus vectors and methods of use. Wilson, James M., et al. 424/93.2; 435/456 A61K048/00 C12N015/861.

☐ 15. 20040126885. 04 Nov 03. 01 Jul 04. Delivery vehicle for recombinant proteins. Cines, Douglas B., et al. 435/455; 435/372 514/44 536/23.1 C12N005/08 C12N015/85.

☐ 16. 20040058320. 21 Dec 01. 25 Mar 04. Reagents and methods for identifying and modulating expression of tumor senescence genes. Roninson, Igor B., et al. 435/6; 435/7.23 C12Q001/68 G01N033/574.

☐ 17. 20040052764. 25 Oct 02. 18 Mar 04. Recombinant aav vectors with aav5 capsids and aav5 vectors pseudotyped in heterologous capsids. Hildinger, Markus, et al. 424/93.2; 435/456 A61K048/00 C12N015/861.

☐ 18. 20040033604. 25 Oct 02. 19 Feb 04. Recombinant lentiviral vectors pseudotyped in envelopes containing filovirus binding domains. Kobinger, Gary, et al. 435/456; 424/93.2 435/235.1 C12N015/867 C12N007/00 A61K048/00.

☐ 19. 20030228282. 25 Apr 03. 11 Dec 03. Adeno-associated virus (AAV) serotype 8 sequences, vectors containing same, and uses therefor. Gao, Guangping, et al. 424/93.2; 435/235.1 435/372 435/456 530/350 536/23.72 A61K048/00 C07H021/04 C12N007/00 C12N015/861 C07K014/075 C12N005/08.

☐ 20. 20030215873. 15 Jul 02. 20 Nov 03. Methods for identifying and using MarR family polypeptide binding compounds. Alekshun, Michael N., et al. 435/7.1; 702/19 G01N033/53 G06F019/00 G01N033/48 G01N033/50.

☐ 21. 20030190617. 06 Mar 02. 09 Oct 03. Optineurin nucleic acid molecules and uses thereof. Raymond, Vincent, et al. 435/6; 435/252.3 435/325 435/368 536/23.2 C12Q001/68 C12N005/08 C07H021/04 C12N001/21.

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☐ 23. 20030139330. 25 Nov 02. 24 Jul 03. Transcription factors containing two potential dna binding motifs. Banham, Alison Hilary, et al. 514/12; 435/199 435/320.1 435/325 435/6 435/69.1 514/44 536/23.2 A61K048/00 A61K038/17 C12P021/02 C12N005/06 C12Q001/68 C07H021/04 C12N009/22.

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☐ 25. 20030119191. 20 Sep 02. 26 Jun 03. Compositions and methods for helper-free production of recombinant adeno-associated viruses. Gao, Guangping, et al. 435/456; 435/235.1 C12N015/861 C12N007/00.

☐ 26. 20030114650. 11 Jan 02. 19 Jun 03. Compositions and methods for affecting virulence determinants in bacteria. Cheung, Ambrose L., et al. 536/23.1; C07H021/02 C07H021/04.

☐ 27. 20030104374. 29 Mar 01. 05 Jun 03. Gene encoding MNR2 and uses thereof. Jessell, Thomas M., et al. 435/6; 435/226 435/320.1 435/368 435/69.1 536/23.2 C12Q001/68 C07H021/04 C12N009/64 C12P021/02 C12N005/06 C12N005/08.

☐ 28. 20030092161. 19 Sep 02. 15 May 03. Compositions and methods for production of recombinant viruses, and uses therefor. Gao, Guangping, et al. 435/235.1; 435/239 435/456 C12N007/00 C12N007/02 C12N015/86.

☐ 29. 20030013189. 17 Sep 01. 16 Jan 03. Compositions and methods useful for non-invasive delivery of therapeutic molecules to the bloodstream. Wilson, James M., et al. 435/320.1; 424/199.1 424/233.1 424/278.1 435/235.1 A61K039/12 A61K039/23 A61K039/235 C12N007/00 C12N007/01 A61K045/00 A61K047/00 C12N015/00 C12N015/09 C12N015/63 C12N015/70 C12N015/74.

☐ 30. 20020197678. 11 Mar 02. 26 Dec 02. Gene encoding MNR2 and uses thereof. Jessell, Thomas M., et al. 435/69.1; 435/183 435/320.1 435/325 536/23.2 C12N009/00 C07H021/04 C12P021/02 C12N005/06.

☐ 31. 20020019050. 05 Apr 01. 14 Feb 02. Compositions and methods for helper-free production of recombinant adeno-associated viruses. Gao, Guangping, et al. 435/456; 435/235.1 C12N015/861 C12N007/01.

☐ 32. 7022519. 20 Sep 02; 04 Apr 06. Compositions and methods for helper-free production of recombinant adeno-associated viruses. Gao; Guangping, et al. 435/325; 435/320.1. C12N15/63 20060101 C12N5/10 20060101 .

☐ 33. 6953690. 20 Sep 00; 11 Oct 05. Compositions and methods for helper-free production of recombinant adeno-associated viruses. Gao; Guangping, et al. 435/325; 424/93.2 424/93.21 435/320.1 435/455 435/456 536/23.1 536/24.1. C12N005/00 C12N015/86 C12N015/00 A01N063/00 C07H021/04 .

☐ 34. 6884618. 02 Apr 02; 26 Apr 05. Identification of the gene causing the mouse scurfy phenotype and its human ortholog. Brunkow; Mary E., et al. 435/320.1; 435/325 435/455 435/69.1 536/23.1 536/23.5. C12N005/00 C12N015/00 C12N015/63 C07H021/02 C07H021/04 .

☐ 35. 6821512. 30 May 02; 23 Nov 04. Compositions and methods for increasing packaging and yield of recombinant adenoviruses using multiple packaging signals. Gao; Guangping, et al. 424/93.2; 424/93.1 424/93.6 435/320.1 435/325 435/455 435/456 435/457 435/69.1. C12N015/861 C12N015/63 C12N015/64 A61K048/00 .

☐ 36. 6793926. 25 May 00; 21 Sep 04. Methods for production of a recombinant adeno-associated virus. Rasty; Siyamak, et al. 424/199.1; 424/184.1 424/204.1 424/233.1 435/235.1 435/320.1 435/455 435/456 435/457 435/69.1 514/44 536/23.1 536/23.72. A61K039/12 .

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☐ 38. 6485966. 05 Apr 01; 26 Nov 02. Compositions and methods for helper-free production of

recombinant adeno-associated viruses. Gao; Guangping, et al. 435/320.1; 435/235.1 435/239 435/325 435/455 435/466 514/44 536/23.1. C12N015/861 C12N007/01 C12N015/09 A61K048/00 C07H021/04 .

☐ 39. 6387656. 29 Sep 98; 14 May 02. Gene encoding MNR2 and uses thereof. Jessell; Thomas M., et al. 435/69.1; 435/252.3 435/320.1 435/325 536/23.5. C12D021/06 .

☐ 40. 6258595. 23 Sep 99; 10 Jul 01. Compositions and methods for helper-free production of recombinant adeno-associated viruses. Gao; Guang-Ping, et al. 435/320.1; 435/239 435/325 435/455 435/466 514/44 536/23.1. C12N015/86 C12N015/00 C12N005/00 C07H021/04 A61K048/00 .

☐ 41. 6187533. 09 Sep 97; 13 Feb 01. Mutations in the diabetes susceptibility genes hepatocyte nuclear factor (HNF) 1 alpha (.alpha.), HNF1.beta. and HNF4.alpha.. Bell; Graeme I., et al. 435/6; 435/91.2. C12Q001/68 C12P019/34 .

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Term	Documents
WINGED	10944
WINGEDS	0
HELIX	93261
HELICES	20279
HELIXES	3695
PROTEIN$	0
PROTEIN	428822
PROTEINA	301
PROTEINAACEOUS	1
PROTEINAAEOUS	1
PROTEINAAGENT	1
(WINGED NEAR HELIX NEAR PROTEIN$).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD.	41

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DOCUMENT-IDENTIFIER: US 20030114650 A1
TITLE: Compositions and methods for affecting virulence determinants in bacteria

Detail Description Paragraph:
[0064] The present invention also provides a detailed three-diminsional (3-D) crystal structure of the SarR protein. The structural data demonstrate that the protein is a member of a new family of winged helix proteins.

Detail Description Paragraph:
[0136] The structure of SarR-MBP fusion protein shows that the SarR dimer is located at the top of the two individual MBP molecules, connected by two flexible loop regions between SarR and MBP. The positions of the MBP molecules suggest that they did not influence the structure of SarR dimer (FIG. 9A). The overall structure of the SarR monomer consists of five .alpha. helices, three short .beta. strands, and several loops (FIG. 9B). The (.alpha.1 helix extends out from the remaining molecule, forming a `L` shaped like structure with a stretch formed from the N-terminal residues of the protein. The .alpha.2 helix follows a 7-residue loop from .alpha.1, and is almost perpendicular to .alpha.1 (85.degree.). The three .beta. strands, .beta.1, .beta.2, and .beta.3, form an anti-parallel bundle, which is slightly twisted. .alpha.5 follows immediately after .beta.3. Between .beta.1 and .beta.2 is a long flexible region (residues 51-79), which has poor electron density in the initial 2Fo-Fc and Fo-Fc maps, containing two helices, .alpha.3 (residue 51-56) and .alpha.4 (residue 63-75) respectively, and a short turn (residue 56-58). These three elements build up a typical helix-turn-helix structural module existing in DNA-binding proteins. Homology alignment of the SarR structure with all available structures shows that the SarR monomer is homologous to winged helix proteins (23), such as transcription regulatory protein mota fragment (PDB code 1bja) with Z score of 8.0, transcriptional repressor smtb activation domain (PDB code 1smt) with Z score of 7.2. Compared to winged helix proteins, "W2" is replaced by a helix (.alpha.5) and the "W1" extends much further in the SarR monomer (FIG. 9B). The above data shows that SarR and its family of proteins are new members of the classic winged helix protein family.

Detail Description Paragraph:
[0144] The SarR dimer was superimposed on the CAP-DNA complex to construct a model for a SarR-DNA complex (FIGS. 13B and C). This model suggests that in addition to interactions of the .alpha.4 helix with the DNA major groove, SarR makes contacts with the DNA minor groove. The loop region between .beta.2 and .beta.3 and part of the two beta strands (W1, a .beta.-hairpin) should be quite flexible in the free SarR structure. Only slight adjustments of their conformations are required to position them to interact intensively with the minor groove of the DNA. Several residues that could be involved in the interactions are highly conserved: Asp 86, Glu 87, and Arg 88 with the side chain of Arg 88 interacting with the DNA phosphate backbone and the side chains of Asp 86 and Glu 87 interacting with bases (FIGS. 13B and C). This loop is too short in CAP and other winged helix proteins to have this minor groove binding function (23). One new member of one of the classes of winged helix proteins (RFX) does make DNA minor groove contacts, but in this case, the wing contacts the major groove and the helix contacted the minor groove (24). Therefore, the predicted SarR type of wing-minor groove interaction appears to be a unique feature of the SarA family of proteins establishing them as a third class of the winged helix family (23,24).

23. Gajiwala, K. S., Burley, S. K. (2000) Current Opinion in Structural Biology, 10:110-116.

[0173] 24. Gajiwala, K. S., Chen, H., Cornille, F., Roques, B. P., Reith, W., Mach, B., Burley, S. K. (2000) Nature 403 (6772):916-21.

First Hit

L1: Entry 23 of 41 File: PGPB Jul 24, 2003

DOCUMENT-IDENTIFIER: US 20030139330 A1
TITLE: Transcription factors containing two potential dna binding motifs

Summary of Invention Paragraph:
[0010] One of the proteins embodying the invention has been designated FOXP1 in line with a new unified nomenclature for the winged helix/forkhead transcription factors (Kaestner, K. H., W. Knuchel and D. E. Martnez, 2000. 14:142-146). This name has been assigned by Dr Daniel Martnez of the Fox Nomenclature Committee on behalf of the HUGO Nomenclature Committee. All further references to this protein will be as FOXP1 and this will be the name which appears in the Genbank accessions AF146696-AF146698 and AF275309. The FOXP1 homologues human cDNA clone YX52E07 (accession AF086040) and mouse QRF1 (accession A49395) have been named FOXP2 and Foxp1 respectively. Collectively these genes define a new subgroup of winged helix proteins. The Fox Nomenclature Index maintained on the Web at http://www.biology.pomona.edu/foxindex.html identifies the FOXP1 gene sequence as submitted by A. Banham.

Detail Description Paragraph:
[0119] A potential open reading frame of 677 amino acids, bordered by stop codons at both the 5' and 3' sides, was encoded by nucleotides 264-2294 of the pAB195 cDNA sequence, which is illustrated in FIG. 2 with the amino acid sequence shown below. There are two potential in-frame methionine ATG initiation codons, the first has purines in both positions -3 and +4 and the second only has a purine at -3. However the second ATG has a better Kozak consensus (GCCA/GCCATGG) (Kozak, M. (1987) J. Mol. Biol. 196: 947-950) than the first because of the occurrence of A at position -3 and G at position -6 (Kozak, M. (1987) Nuc. Acids Res. 15: 8125-8148). The upstream non-coding region has a higher GC-rich content than the coding sequence which is frequently associated with the 5' end of genes. At least two other winged helix proteins, WIN (Yao, K.-M., Sha, M., Lu, Z. and Wong, G. G. (1997) J. Biol. Chem. 272: 19827-19836) and AF6q21 (Hillion, J., Le Coniat, M., Jonveaux, P., Berger, R. and Bernard, O. A. (1997) Blood 90: 3714-3719), also start with two ATG codons and both use the second, further supporting the hypothesis that translation starts at the second ATG. Interestingly upstream ATG codons occur in fewer than 10% of vertebrate mRNAs-at-large although a notable exception are oncogene transcripts, two-thirds of which have ATG codons preceding the start of the major open reading frame (Kozak, M. (1987) Nuc. Acids Res. 15: 8125-8148).

Detail Description Paragraph:
[0123] Another interesting feature of the winged helix/zinc finger protein is the prediction that there are two regions between aa 124-155 and aa 344-369 which have the potential to form coiled coils (Lupas, A., Van Dyke, M. and Stock, J. (1991) Science 252: 1162-1164). These motifs are important in a wide range of transcription factors (and other proteins) where they mediate protein-protein interactions and act as dimerization motifs (reviewed in Baxevanis, A. D. and Vinson, C. R. (1993) Curr. Opin. Genet. Dev. 3: 278-285). The coiled coil motifs identified in this winged helix/zinc finger protein are not a characteristic of the winged helix family in general as our analysis of a number of other winged helix proteins (HNF-3.alpha., Genesis, FKHRLI, FREAC-1 and HFH-4) did not predict the presence of these motifs.

L1: Entry 23 of 41 File: PGPB Jul 24, 2003

PGPUB-DOCUMENT-NUMBER: 20030139330
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030139330 A1

TITLE: Transcription factors containing two potential dna binding motifs

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Banham, Alison Hilary	Headington Oxford		GB
Cordell, Jacqueline Loelia	Headington Oxford		GB
Jones, Margaret	Headington Oxford		GB

APPL-NO: 10/148662 [PALM]
DATE FILED: November 25, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	9928543.9	1999GB-9928543.9	December 2, 1999
GB	0017042.3	2000GB-0017042.3	July 11, 2000

PCT-DATA:

DATE-FILED	APPL-NO	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
Dec 1, 2000	PCT/GB00/04590				

INT-CL-PUBLISHED: [07] A61 K 48/00, A61 K 38/17, C12 P 21/02, C12 N 5/06, C12 Q 1/68, C07 H 21/04, C12 N 9/22

US-CL-PUBLISHED: 514/12; 514/44, 435/6, 435/69.1, 435/320.1, 435/325, 435/199, 536/23.2
US-CL-CURRENT: 514/12; 435/199, 435/320.1, 435/325, 435/6, 435/69.1, 514/44, 536/23.2

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

There is disclosed an isolated protein comprising (i) a winged helix motif which has the potential capability of binding to nucleic acid and (ii) a Cys.sub.2-His.sub.2 zinc finger motif which also has nucleic acid binding capability and nucleic acid molecules encoding therefor.

First Hit

L1: Entry 16 of 41 File: PGPB Mar 25, 2004

DOCUMENT-IDENTIFIER: US 20040058320 A1
TITLE: Reagents and methods for identifying and modulating expression of tumor senescence genes

Detail Description Paragraph:
[0069] One class of genes that are differentially expressed in doxorubicin-treated HCT 116 cells are genes encoding known or putative transcription factors or cofactors. Genes for several known or putative transcription factors and cofactors show altered regulation in the senescent HCT116 cells. One of the downregulated transcription factors is winged helix protein HFH-11 (Trident), a positive regulator of DNA replication, that is specifically expressed in cycling cells (Ye et al., 1999, Mol. Cell. Biol. 19: 8570-8580). Several upregulated transcription factors are related to the AP-1 family, which mediates cellular responses to various mitogenic signals, interferons and different forms of stress (Wisdom, 1999, Exp. Cell. Res. 253: 180-185). These include c-Jun and two other basic leucine zipper proteins, XBP-1 (structurally related to c-Jun) and ATF3 that dimerizes with c-Jun. Sustained upregulation of ATF3 mRNA and protein in senescent cells is surprising, since induction of this stress-responsive factor is usually transient (over hours), due to the ability of ATF3 to inhibit its own transcription (Wolfgang et al., 2000, J. Biol Chem. 275: 16865-16870). Another induced transcription factor is ELF-1, a member of Ets family of helix-loop-helix proteins that are known to interact functionally, and possibly physically, with AP-1 (Wisdom, ibid.).

First Hit

L1: Entry 11 of 41 File: PGPB Jan 6, 2005

DOCUMENT-IDENTIFIER: US 20050003386 A1
TITLE: Methods and compositions for detection and analysis of polynucleotide-binding protein interactions using light harvesting multichromophores

Detail Description Paragraph:
[0082] Any protein which can bind to a target polynucleotide of interest can be employed in the methods disclosed. Non-limiting examples of PBPs include DNA-binding proteins including transcription factors, splicing-factors, poly(A) binding proteins, chromatin components, viral proteins, proteins which detect viral infection, replication factors, and proteins involved in mitotic and/or meiotic cell division. RNA-protein interactions mediate important cellular processes including transcription, posttranscriptional modifications, RNA splicing, and translation.sup.37,38,39,40. The replication cycle of many pathogenic viruses, such as the human immunodeficiency virus type 1 (HIV-1).sup.41, picornaviruses.sup.42 and influenza viruses.sup.43, rely on specific RNA-protein interactions. The specificity of such interactions can be used as the basis for sequence specific sensors for utility in medical diagnostics and genomic studies. Exemplary polynucleotide binding proteins include zinc-finger proteins, homeodomain proteins, winged-helix (forkhead) proteins, leucine-zipper proteins, helix-loop-helix proteins, helix-turn-helix proteins, and histone-like proteins.

First Hit

L1: Entry 4 of 41 File: PGPB Oct 27, 2005

PGPUB-DOCUMENT-NUMBER: 20050239704
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050239704 A1

TITLE: Compositions and methods for affecting virulence determinants in bacteria

PUBLICATION-DATE: October 27, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Cheung, Ambrose L.	Hanover	NH	US
Manna, Adhar	Lebanon	NH	US
Zhang, Gongyi	Denver	CO	US

APPL-NO: 11/063308 [PALM]
DATE FILED: February 22, 2005

RELATED-US-APPL-DATA:
Application 11/063308 is a continuation-of US application 10/043539, filed January 11, 2002, PENDING
Application is a non-provisional-of-provisional application 60/261233, filed January 12, 2001,
Application is a non-provisional-of-provisional application 60/261607, filed January 12, 2001,
Application is a non-provisional-of-provisional application 60/289601, filed May 8, 2001,

INT-CL-PUBLISHED: [07] A61 K 38/16, C12 Q 1/68, C07 H 21/04, C12 N 15/74, C12 N 1/21, C07 K 14/31

US-CL-PUBLISHED: 514/012; 536/023.2, 435/006, 435/069.3, 435/252.3, 435/471, 530/350
US-CL-CURRENT: 514/12; 435/252.3, 435/471, 435/6, 435/69.3, 530/350, 536/23.2

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

A novel sarR gene and sarR gene product which down regulates the expression of sarA and the resulting virulence determinants in Staphylococcus aureus is provided. Methods for modulating the expression of sarA and virulence determinants are also provided. A preferred embodiment of the present invention provides structural information relating to the gene product and enables the identification and formulation of lead compounds and reducements for treating and preventing infections by S. aureus and related bacteria.

[0001] This application claims priority to U.S. Provisional Application Ser. No. 60/261,233, filed Jan. 12, 2001, U.S. Provisional Application Ser. No. 60/261,607,

filed Jan. 12, 2001, and U.S. Provisional Application Ser. No. 60/289,601, filed May 8, 2001. These applications are hereby incorporated by references herein in their entirety.

PGPUB-DOCUMENT-NUMBER: 20050239704
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050239704 A1

TITLE: Compositions and methods for affecting virulence determinants in bacteria

PUBLICATION-DATE: October 27, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Cheung, Ambrose L.	Hanover	NH	US
Manna, Adhar	Lebanon	NH	US
Zhang, Gongyi	Denver	CO	US

US-CL-CURRENT: 514/12; 435/252.3, 435/471, 435/6, 435/69.3, 530/350, 536/23.2

CLAIMS:

What is claimed is:

1. A nucleic acid sequence which regulates the expression of virulence determinants in gram positive bacteria.

2. The nucleic acid sequence of claim 1, wherein the bacteria is a Staphylococcus species.

3. The nucleic acid sequence of claim 2, wherein the bacterium is Staphylococcus aureus.

4. The nucleic acid sequence of claim 1 comprising the sequence as essentially set forth in SEQ ID NO: 1.

5. A vector comprising the nucleic acid sequence of claim 4.

6. A host cell comprising the vector of claim 5.

7. A method for identifying putative agents which inhibit growth and infectivity of bacteria comprising identifying agents which enhance the expression of the nucleic acid sequence of claim 1 or the activity of a polypeptide encoded thereby.

8. The method of claim 7 wherein the bacterium is Staphylococcus species.

9. The method of claim 8 wherein the bacterium is Staphylococcus aureus.

10. A method of inhibiting growth and infectivity of bacteria comprising contacting the bacteria with an agent which enhances the expression of the nucleic acid sequence of claim 1 or the activity of a polypeptide encoded thereby.

11. The method of claim 10 wherein the bacterium is a Staphylococcus species.

12. The method of claim 10 wherein the bacterium is Staphylococcus aureus.

13. A pharmaceutical composition for use as an anti-bacterial agent comprising an agent which enhances the expression of the nucleic acic sequence of claim 1 or the activity of a polypeptide encoded thereby and a pharmaceutically acceptable vehicle.

14. The composition of claim 13 which is an anti-bacterial agent against a Staphylococcus species.

15. The composition of claim 13 wherein the bacterium is Staphylococcus aureus.

16. An isolated polypeptide which regulates the expression of virulence determinants in gram positive bacteria.

17. A polypeptide encoded by the nucleic acid sequence essentially as set forth in SEQ ID NO: 2.

18. The isolated polypeptide of claim 16 wherein the bacterium is Staphylococcus species.

19. The isolated polypeptide of claim 18 wherein the bacterium is Staphylococcus aureus.

20. A kit for identifying the presence of a sarR gene or sarR gene product comprising a means for analyzing a biological sample for the presence of the sarR gene or the sarR gene product.

21. A method of treating a mammal suffering from or susceptible to a gram positive bacterial infection comprising administering a compound capable of selective occupation of a sarA promoter receptor.

22. A method of screening for lead compounds which inhibit the expression of virulence determinants in gram positive bacteria comprising identifying chemical entities having structural similarities to the SarR protein sufficient to allow said chemical entities to form heterodimers with SarA protein.

23. A method of screening for lead compounds which inhibit the expression of virulence determinants in gram positive bacteria comprising identifying chemical entities having structural similarities to the SarR protein sufficient to allow said chemical entities to bind sarA promoter receptors.

24. A pharmaceutical composition comprising a compound identified by the screening method of claim 22 or 23.

25. A pharmaceutical composition comprising-a compound identified by the screening method of claim 22 or 23, wherein said composition is capable of inhibiting the expression of sarA.

26. A pharmaceutical composition for the treatment of gram positive bacteremia comprising a SarR agonist.

27. A pharmaceutical composition comprising a compound that binds to the P1 promoter region of a sarA gene.

WEST Search History

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☐	L2	forkhead or fork-head	656
☐	L3	L2.ti,ab,clm.	84

END OF SEARCH HISTORY

First Hit Fwd Refs

L7: Entry 5 of 11 File: USPT Mar 2, 2004

DOCUMENT-IDENTIFIER: US 6699662 B1
TITLE: Inhibitors of staphylococcus SarA protein function involved in the expression of staphylococcal virulence factors and the use thereof in treating staphylococcal infections

Abstract Text (1):
The present invention relates to inhibitors of Staphylococcus SarA protein function involved in the expression of staphylococcal virulence factors and the use of these inhibitors to treat and prevent staphylococcal infections in subjects. Particularly, the inhibitors act to interfere with the binding of the SarA protein to its binding site(s). The selection of specific inhibitors of the SarA protein is made possible as a result of the identification of the binding sites of SarA protein on at least a portion of the agr (accessory gene regulator) gene, a gene that like the sar (staphylococcal accessory regulator) gene, plays a role in the virulence of Staphylococcus. The present invention also is directed to a method of designing, synthesizing and identifying inhibitors of Sar A function and its role in the expression of staphylococcal virulence factors.

Brief Summary Text (3):
The present invention relates to inhibitors of Staphylococcus SarA protein function involved in the expression of staphylococcal virulence factors and the use of these inhibitors to treat and prevent staphylococcal infections in subjects. Particularly, the inhibitors act to interfere with the binding of the SarA protein to its binding site(s). The selection of specific inhibitors of the SarA protein is made possible as a result of the identification of the binding sites of SarA protein on at least a portion of the agr (accessory gene regultor) gene, a gene that like the sar (staphylococcal accessory regulator) gene, plays a role in the virulence of Staphylococcus.

Brief Summary Text (4):
There is a great and urgent need among infectious-disease specialists, who have begun seeing one of their worst nightmares come true. They may be losing their last line of defense against the dangerous pathogen Staphylococcus aureus (S. aureus), which causes infections ranging from skin abscesses to such life-threatening conditions as pneumonia, endocarditis, septicemia, and toxic shock syndrome. Roughly one-third of the strains currently isolated from patients who acquire S. aureus infections while hospitalized are resistant to all antibiotics but one, vancomycin and now resistance to that antibiotic is cropping up. The present invention provides a new approach to combating S. aureus that may sidestep the organism's ability to develop resistance.

Brief Summary Text (5):
Despite intensive research efforts over the past 50 years, Staphylococcus, particularly Staphylococcus aureus, remains a serious threat to human health. In fact, recent reports describe clinical isolates with reduced susceptibility to vancomycin. Therefore, S. aureus represents a bigger threat to human health now, than at any time since the pre-antibiotic era.

Brief Summary Text (6):
Staphylococcus is an opportunisitic bacteria that takes advantage of immunocompromised subjects and may become pathogenic in these subjects. There are

approximately thirty-two species of Staphylococcus with only three consistently causing human disease. S. aureus is clearly the most prominent disease causing species, followed by S. epidermidis, and in a distant third is S. saprophyticus. S. epidermidis is becoming more prominent as a disease causing species because it causes infections of in-dwelling medical devices. As a result, researchers are looking more carefully at S. epidermidis, and as a result of this research, have found homologs of both the sar and agr genes in S. epidermidis. Fluckiger, U., et al. (1998). Otto,M., et al. (1998), respectively.

Brief Summary Text (7):
S. aureus can cause a diverse array of diseases ranging from relatively superficial infections of the skin (boils) to infections of the eye (endopthalmitis) to life threatening osteomyelitis, endocarditis and toxic shock syndrome (reviewed by Projan and Novick, 1997). S. aureus is armed with a large battery of virulence factors that enable it to colonize a human host and cause a variety of disease states (reviewed by Projan and Novick, 1997). Nosocomial infections are of particular concern for two reasons. The first is that the majority of life-threatening infections arise in the hospital environment. For example, while the frequency of S. aureus infections incurred during orthopedic or cardiac implant surgery is steady, the overall number of infections has risen dramatically in the past decade. This is largely due to the increase in the frequency of these procedures. S. aureus has an amazing capacity to colonize in-dwelling prosthetic devices. The second reason for increased concern of S. aureus infections is that strains of methicillin-resistant Staphylococcus aureus (MRSA) are endemic in hospitals. Moreover, strains with some resistance to vancomycin emerged in the United States in 1997 (Tenover et al., 1998; Smith et al., 1999; Sieradzki et al., 1999). Therefore, the need for new, effective treatments for this drug resistant pathogen is urgent.

Brief Summary Text (8):
The variety of virulence factors expressed by S. aureus contribute to a highly efficient system for survival. Early in the infection, surface proteins are predominantly expressed. Protein A and the adhesins (e.g., collagen, fibronectin) are representative surface proteins that solve two problems for the S. aureus cell. First, they bind to extracellular matrix components and anchor the cell to the host tissue. Second, they provide a host protein camouflage which helps the infecting cell elude the host's immune system. The nascent colony increases in size until a critical number of cells is achieved (quorum) and a switch is thrown to re-organize the expression of virulence factors from surface proteins to exoproteins. These latter factors contribute to sequestration of the colony within a protective biofilm and enzymatic degradation of host tissue with an army of digestive enzymes, such as nucleases, lipases and proteases, which eventually result in an abscess. These enzymes accomplish two important functions or the bacterium: (1) allowing space for growth of the colony by getting rid of host tissue and (2) digested host tissue is assimilated by the bacterial cells for growth. Deep-seated abscesses, such as those found in staphylococcal ostemyelitis and endocarditis, often require surgical intervention to remediate the disease. It is important to note that this phenotypic switching process can be largely recapitulated in the laboratory environment, with surface protein expression occurring in the early log phase of a culture's growth and exoprotein expression occurring late in log and into the stationary phases of growth.

Brief Summary Text (9):
The potency of this pathogen can be attributed to the coordinated, temporally-regulated expression of a wide array of virulence factors. Early in infection expression of surface proteins predominates, e.g., the collagen and fibronectin adhesins and protein A. The surface proteins allow the organism to attach to host tissues and evade the immune system. However, when the concentration of S. aureus cells at the site of infection becomes high, surface protein expression is reduced and exoprotein expression increases. The temporal regulation of surface proteins

and exoproteins can be recapitulated in laboratory culture growth models, where early log phase growth represents an early infection and stationary phase represents late infection. Using this model system and classical genetics, two major pleiotropically-acting regulatory loci that govern temporal expression of surface proteins and exoproteins have been identified: agr, for accessory gene regulator (Recsei et al., 1986; Morfeldt et al., 1988; Peng et al., 1988) and sar, for staphylococcal accessory gene regulator (Cheung et al., 1992; Cheung and Projan, 1994). Mutations in these loci result in aberrant regulation of most virulence factors (e.g., lipase, coagulase, .alpha.-toxin, adhesins, etc), which is reflected in diminished virulence in animal models of staphylococcal disease (Projan and Novick, 1997).

Brief Summary Text (10):
A scheme depicting the agr locus and its encoded proteins is shown in FIG. 1. Divergent promoters (P2 and P3), separated by approximately 180 bp, are responsible for transcription of the agrBDCA operon and RNAIII/hld operon (Morfeldt et al., 1996). The four Agr proteins combine to make a quorum-sensing system that is homologous to many two-component signal transduction systems found in prokaryotic organisms (Ji et al., 1997). AgrB is a cell membrane-bound transporter/processor of the AgrD peptide. AgrD is a 46 amino acid peptide that is cleaved to an octapeptide pheromone, exported by AgrB, and specifically recognized by (Ji et al., 1997) the AgrC membrane-bound receptor. The AgrD octapeptide pheromone allows an S. aureus cell to signal its presence to other cells in the growing colony. As the colony grows, the concentration of pheromone (Agr D) increases and reaches a particular level. AgrC, also an integral membrane protein, is activated by pheromone binding. AgrC is thought to be a kinase that acts on AgrA by initiating a signal transduction pathway that is believed to include AgrA. Whereas the exact mechanism of AgrA action is unknown, it is important for up-regulation of virulence gene expression and is thought to activate expression of the agr operon (RNAII) and the divergently expressed RNAIII. It is clear, however, from the work of Arvidson's group (Morfeldt et al., 1996) that AgrA does not bind DNA either in the presence or absence of SarA. Mutations in any of the agr open reading frames (ORFs A, B, C, D) eliminate the up-regulation of RNAII and RNAIII expression (Novick et al., 1995). Additionally, agrA mutants have dramatically reduced virulence in animal models of staphylococcal arthritis, osteomyelitis, endocarditis and endopthalmitis (Abdelnour et al., 1993; Cheung et al., 1994a; Gillaspy et al., 1995; Booth et al., 1995). RNAIII is a regulatory RNA species, the function of which is not completely clear. However, there is evidence that RNAIII directly regulates expression of some S. aureus virulence genes by an anti-attenuation mechanism (Novick et al., 1993; Saravia-Otten et al., 1997).

Brief Summary Text (12):
Mutations in agr leads to decreased expression of exoprotein virulence factors and significantly reduced virulence in animal models of staphylococcal arthritis, endocarditis, osteomyelitis and endopthalmitis (Abdelnour et al., 1993; Cheung et al., 1994a; Gillaspy et al., 1995; Booth et al., 1995, respectively). Inhibition of the agr quorum-sensing/virulence gene activating system is a goal of the present invention. Since agr is activated by a transcriptional regulator, SarA, the present invention is directed to inhibiting this protein.

Brief Summary Text (13):
The second regulatory locus, sar, encodes a 14.4 kDa protein: SarA, also depicted in FIG. 1. Mutations in sar, like those in agr, lead to dramatically decreased virulence in animal models of staphylococcal disease (Cheung et al., 1994a). Interestingly, agr.sup.-, sar.sup.- double mutants are less virulent than either of the single mutants in staphylococcal endocarditis, endophthalmitis and osteomyelitis (Cheung et al., 1994b; Booth et al., 1997; Gillaspy, et al., unpublished). Presumably, this phenotype is because SarA regulates expression of both transcripts in the agr locus (agr and RNAIII; Cheung et al., 1997) and SarA also regulates virulence factor genes that fall outside of agr control. For

example, the cna gene, encoding the collagen adhesin, is not affected by mutations in the agr locus, but is under sar control (Gillaspy et al., 1998; Blevins et al., 1999). agr mutants do not have altered sar mRNA accumulation, whereas agr mRNA expression is dramatically affected by sar mutations (Cheung et al., 1997; Gillaspy and Smeltzer, unpublished). Specifically, there is a significant diminution of agr mRNA and nearly a complete loss of RNAIII in the sar strain ALC136 when compared to the wild type strain RN6390 (Cheung et al., 1997). The same observation has been made in clinical isolates in which the sar gene has been mutated (Gillaspy and Smeltzer, unpublished).

Brief Summary Text (16):
Production of the three distinct transcripts arising from the sar operon are regulated temporally (Bayer et al., 1996; Blevins et al., 1999). However, all three transcripts include the SarA ORF. Like agrA mutations, transposon insertions in the SarA ORF also eliminate induction of RNAII and RNAIII in late phase growth and result in reduced staphylococcal virulence in animal models of disease (Cheung et al., 1994 a and b; Booth et al., 1997). In seminal biochemical work in this area, SarA was shown to be a DNA-binding protein that is capable of binding DNA fragments containing cis regulatory elements for the promoters of both the agr operon (RNAII, P2 promoter) and the RNAIII operon (P3 promoter) (Morfeldt et al., 1996). Heptad repeats were identified upstream of both P2 and P3 promoters and were proposed to be SarA binding sites (see FIG. 2 and FIG. 3). A DNA fragment containing the RNAIII gene and 93 bp upstream of the transcription start site, including the heptad repeats, was sufficient for regulated expression of RNAII (see pEX085 in FIG. 3). Furthermore, removal of the distal half of the sequences upstream of the P3 promoter, including one heptad, eliminated appropriate expression of RNAIII (see pEX082 in FIG. 3). In addition, a synthetic DNA fragment including the repeats was bound by SarA in electrophoretic mobility shift assays (EMSA) in vitro using S. aureus extracts and was used successfully to purify SarA from extracts by DNA-affinity chromatography (Morfeldt et al., 1996).

Brief Summary Text (20):
The present invention approaches the treatment of staphylococcal virulence and infection differently than previous publications by the inhibiting the activation of agr gene expression by inhibiting SarA function resulting in the inhibition of the expression of staphylococcal virulence factors. The present method of treatment provides a way to attenuate staphylococcal virulence which is believed to be more widely applicable than the Balaban inhibitor. This is so because SarA and its target DNA sequences cis to agr do not suffer as much strain variability as the Balaban inhibitor. Moreover, since the agr locus also includes RNAIII, a known regulator of virulence gene translation, inhibiting agr gene expression will have a more profound effect than inhibition of the quorum sensing system alone as disclosed by Balaban.

Brief Summary Text (21):
The present invention provides a novel method of treating staphylococcal diseases by interfering with the production of virulence factors, which in turn, prevents the Staphylococcus species from becoming a potent pathogen. The present invention is directed to designing, synthesizing and identifying potent inhibitors of SarA function and using these inhibitors to treat staphylococcal infections.

Brief Summary Text (23):
The present invention is directed to inhibitors of staphylococcal SarA protein function involved in the expression of staphylococcal virulence factors and the use of these inhibitors to treat and prevent staphylococcal infections in subjects. Particularly, the inhibitors act to interfere with the binding of the SarA protein to its binding site(s). The selection of specific inhibitors of the SarA protein is made possible as a result of the identification of the binding sites of SarA protein on at least a portion of the agr (accessory gene regulator) gene, a gene that like the sar (staphylococcal accessory regulator) gene, plays a role in the

virulence of staphylococci species.

Brief Summary Text (24):
The present invention further is directed to a method of identifying inhibitors of SarA function involved in the expression of staphylococcal virulence genes comprising a) contacting a candidate inhibitor with at least one SarA binding site of the agr locus in solution to allow the binding reaction to equilibrate for a sufficient period of time; and b) assessing the binding of said candidate inhibitor to the SarA binding site of the agr locus.

Brief Summary Text (25):
The present invention further is directed to a method of identifying inhibitors of SarA function involved in the expression of staphylococcal virulence genes comprising a) contacting a candidate inhibitor with SarA in solution to allow the candidate inhibitor to affect the ability of SarA to bind to at least one SarA binding site of the agr locus; b) contacting said solution of step a) with at least one SarA binding site of the agr locus either simultaneously with the contact of said inhibitor and the SarA or subsequently to the contacting of the inhibitor and the SarA; and c) assessing the inhibition of the candidate inhibitor on the SarA binding to the SarA binding site of the agr locus.

Detailed Description Text (2):
The present invention combines genetic, biochemical and structural studies to define the mechanism by which SarA controls expression of the genes in the agr locus. The agr locus includes agrDCBA, RNAIII/hld (See FIG. 1). This work, in turn, allows the design and synthesis of inhibitors of SarA arnd the testing of the effects of these inhibitors of SarA and its regulated virulence genes with a major emphasis on the development of novel anti-staphylococcal therapeutics to combat the onslaught of drug resistant staphylococcal pathogens.

Detailed Description Text (3):
The present invention is directed to inhibitors of SarA function involved in the expression of staphylococcal virulence genes and a method of treating a staphylococcal infection comprising administering to a subject having a staphylococcal infection at least one inhibitor of SarA function involved in the expression of staphylococcal virulence genes. The inhibitor is designed to interfere with SarA mediated activation of the agr locus and more specifically the inhibitor interferes with the binding of SarA to at least a portion of the agr locus. The portion of the agr locus is composed of greater than about 70% adenosine-thymidine (A-T) nucleotides, more preferably at least about 75% A-T nucleotides, and most preferably between about 79% and 89% A-T nucleotides. More specifically, the inhibitor interferes with the binding of SarA to at least a portion of the agr locus depicted in FIG. 3. The inhibitor is designed to interfere with the binding of SarA to at least one of the portions of the agr locus depicted in FIG. 3 that contains a nucleotide sequence selected from the group consisting of at least the nucleotide sequences in the A1 and A2 boxes, at least the nucleotide sequences in the B1 and B2 boxes and at least the nucleotide sequences in the C1 and C2 boxes. Further, the inhibitor also interferes with the binding of SarA to the intervening nucleotide sequences between the A1 and A2 boxes when the inhibitor binds to the nucleotide sequence in the A1 and A2 boxes, to the intervening nucleotide sequences between the B1 and B2 boxes when the inhibitor binds to the nucleotide sequences in the B1 and B2 boxes or to the intervening nucleotide sequences between the C1 and C2 boxes when the inhibitor binds to the nucleotide sequences in the C1 and C2 boxes. The most preferred inhibitors of SarA binding bind to at least a portion of the agr locus, and the most preferred inhibitors are oligonucleotide analogs that utilize the Watson & Crick basepairing to bind to nucleic acids, such as peptide nucleic acid molecules, DNA molecules, RNA molecules, phosphothiolate oligonucleotides, and anti-sense oligonucleotides. Hairpin polyamides are also preferred inhibitors of the present invention. Any molecules that can enter a staphylococcus and interfere with the SarA function in

the expression of staphylococcal virulence factors are encompassed by the present invention. Such molecules can be synthesized, tested and identified utilizing the methods disclosed in the present invention. The inhibitor is admixed with an acceptable carrier, such as a pharmaceutically acceptable carrier for administration to the subject having a staphylococcal infection. The pharmaceutically acceptable carrier may contain preservatives and other non-immunogenic additives, according to methods well known in the art. See, e.g. Reminington's Pharmaceutical Sciences: Drug Receptors and Receptor Theory, (1990). The carrier may contain additives that are known to facilitate the movement of the inhibitor into the staphylococci without adversely affecting the subject treated. Such additives are known to skilled persons or can be selected using known methods. These additives facilitate the movement into the cell of inhibitors that do not readily cross cell membranes, and peptides are known additives that facilitate such movement across the cell membranes.

Detailed Description Text (4):
The inhibitors of the present invention are intended for use in treating all staphylococcal infections. As discussed above, S. aureus, S. epidermidis and S. saprophyticus are the three disease causing species. It is known that S. aureus and S. epidermidis have homologs of both the sar and agr genes. The sequences of these genes in other species of staphylococci are used to prepare inhibitors of SarA function in these species. Likewise SarA itself is used to develop inhibitors that inactivates SarA so that it cannot bind to the agr locus.

Detailed Description Text (6):
The present invention also is directed to a method of identifying inhibitors of SarA function involved in the expression of staphylococcal virulence genes comprising: a) contacting a candidate inhibitor with at least one SarA binding site of the agr locus in solution to allow the binding reaction to equilibrate for a sufficient period of time; and b) assessing the binding of said candidate inhibitor to said SarA binding site of the agr locus. (See FIG. 15 for an example of the results of this method.) The method further comprises the addition of SarA to the solution of step a) simultaneously with the inhibitor and the SarA binding site, and then an assessment of the binding affinity of the candidate inhibitor relative to the binding affinity of the SarA to the SarA binding site of the agr locus. Alternatively, the addition of SarA to the solution of step a) is sequentially with the inhibitor and the SarA binding site, and then the assessment of the binding affinity of said candidate inhibitor relative to the binding affinity of said SarA to the SarA binding site of the agr locus. (See FIG. 16 for an example of the results of this method showing the results with and without the addition of SarA.)

Detailed Description Text (10):
Additionally, the present invention includes a method of identifying inhibitors of SarA function involved in the expression of staphylococcal virulence genes comprising a) contacting a candidate inhibitor with SarA in solution to allow the candidate inhibitor to affect the ability of SarA to bind to at least one SarA binding site of the agr locus; b) contacting the solution of step a) with at least one SarA binding site of the agr locus either simultaneously with the contact of the inhibitor and the SarA or subsequently to the contact of theinhibitor and the SarA; and c) assessing the inhibition of the candidate inhibitor on the SarA binding to the SarA binding site of the agr locus. This method allows the assessment of the inhibitor directly on the SarA rather than the effect of the inhibitor on the SarA binding site. This method uses the assessment of binding of the inhibitor to the SarA binding site by an electrophoretic mobility shift assay. The SarA binding site of the agr locus used in this method is preferably a nucleotide sequence selected from the group consisting of at least the nucleotide sequences in the A1 and A2 boxes, at least the nucleotide sequences in the B1 and B2 boxes, at least the nucleotide sequences in the C1 and C2 boxes and a combination therof as depicted in FIG. 3.

Detailed Description Text (11):
The present invention targets the expression of genes in the agr locus directly, by inhibiting SarA-mediated activation. This approach offers advantages over known approaches because SarA is a pleiotropically-acting regulator that controls another pleiotropically-acting regulator. By inhibiting SarA, agr will be inhibited as well as any other genes under SarA control (e.g., collagen adhesin, cna, Gillaspy et al., 1998). Synthetic molecules that inhibit protein-DNA interactions have been developed and show tremendous promise for pharmaceutical applications. These molecules include peptide nucleic acid (PNA, Corey, 1996) and hairpin polyamides (HP, White et al., 1998). Both PNA and the HP bind dsDNA with very high specificity and affinity. These molecules have great potential for anti-staphylococcal therapies once appropriate targets are identified. Molecules like PNAs and HPs that are designed to bind in the agr regulatory region and inhibit SarA-mediated activation of genes in the agr locus are considered to be inhibitors of SarA function within the meaning of the present invention. Because of the high affinity (sub-nanomolar K.sub.D), very low amounts of the drug or SarA function inhibitor needs be used in a subject in need of treatment. Furthermore, if the subject is to receive a prosthetic device (e.g., heart valve or hip), the drug could be used prophylactically to inhibit S. aureus colonization. The inhibitor can be administered prior to surgery or can be impregnated in beads for slow release and packed around a prosthetic hip device for a while for local administration. Techniques are known for impregnating beads or substrates with drugs. These beads or substrates may be biodegradable.

Detailed Description Text (12):
The present invention is premised upon the determination of the interactions of SarA with the P2-P3 regulatory region, the determination of the mechanism by which SarA regulates virulence gene expression in Staphylococcus, and the biochemical characterization of SarA and its interaction with DNA. In the present invention, a full-length SarA in E. coli was expressed without heterologous fusions and purified to homogeneity. It was determined that SarA was a dimer in the presence or absence of DNA and was comprised primarily of .alpha.-helices. The combined results of DNaseI footprinting and quantitative EMSA experiments indicate that three SarA binding sites exist. Two of the footprints overlap elements of the P2 and P3 promoters. All of the protected sequences included portions of the heptad repeats described by Morfeldt et al. (1996). One SarA dimer was found to bind each binding site with very high affinity. Three dimers bind the entire region and produce an unusual laddering pattern in electrophoretic mobility shift assays (EMSAs).

Detailed Description Text (54):
The present invention is based on the determination of the mechanism of SarA regulation of the two operons in the agr locus and developing inhibitors of that regulation. Based on the strain-to-strain variation observed by Novick and colleagues (Ji et al., 1997), it was a concern that the inhibitors would not work on all clinically-relevant strains. To address this issue, the SarA structural gene and agr enhancer region from thirty genetically distinct strains of Staphylococcus aureus, most of which are clinical isolates, were cloned and sequenced (Smeltzer et al., 1996; Smeltzer et al., 1997). Briefly, the regions of interest were amplified by PCR and cloned into the pTOPO-CR2.1 vector. Several independent clones were sequenced (both strands) from each strain and compared to strain DB for differences. With regard to the SarA coding region, the only changes were observed in strain RN6390: all of the clinical isolates were identical to DB. The agr enhancer region was identical in all strains tested. This result is very important as it supports the utility of the present method of treatment against a broad scope of staphylococcal strains and supports the effectiveness of an inhibitor of SarA in the clinical setting.

Detailed Description Text (75):
Structural information is important for the rational design of anti-staphylococcal inhibitors. Towards this end, two types of structural determinations are pursued.

The first is the traditional x-ray crystallographic approach. Protein or protein and DNA are brought to high concentration, crystallized and the structures are determined from the pattern of x-ray diffraction. This approach typically yields high quality structures at atomic resolution (2.5-1.5 .ANG.). The present invention has already provided a 1.7 ansgstrom data set for SarA alone and crystals of SarA-DNA that diffract to 2.5 angstroms. The second approach is relatively new in biological structure science, atomic force microscopy or AFM. AFM has been pioneered by Dr. Carlos Bustamante and has made a tremendous impact on the field of transcriptional control in the past few years (reviewed in Bustamante and Rivetti, 1996). In AFM, macromolecular species are visualized on mica surfaces in 3-dimensions. Thus, the binding site for SarA can be observed directly, as well as any conformational changes SarA may induce in the DNA. AFM makes it possible to visualize ternary complexes of SarA, P3 and RNA polymerase.

Detailed Description Text (90):
The present invention is based upon the knowledge that pleiotropically-acting regulatory factors are highly attractive targets for novel anti-staphylococcal therapies. Selecting inhbitors that interfere with SarA function which in turn affects the interaction of SarA and agr is particularly appealing, because one can inhibit both SarA and agr gene products at the same time. The design of effective inhibitors of the SarA-mediated activation of agr gene expression requires a detailed understanding of the mechanism of SarA regulation.

Detailed Description Text (100):
Blocking a cis-acting binding site for SarA with a small molecule inhibits SarA-mediated gene activation. In the present invention, the binding sites are those identified already herein and in Rechtin et al, (1999) and those that are cis to the P3 promoter. Among the small molecules that have the desired properties, peptide nucleic acid (PNA) and hairpin polyamides (HP) have received the most attention and show the most promise as pharmacological agents (e.g., Gottesfeld et al., 1997; Corey 1997; White et al., 1998; Good and Nielsen, 1998; Dickinson et al., 1998). Based on the analysis of the cis regulatory sites discussed above, appropriate test PNA and HP molecules for use as anti-staphylococcal agents have been designed, synthesized and tested.

Detailed Description Text (102):
As discussed above, PNA molecules alone have one documented drawback, they do not cross membranes well. Currently, a tremendous amount of effort is being spent in academic and industrial settings to derive "carriers" for PNA. For example, one research group has published increased membrane passage by PNA-peptide conjugates (Hamilton et al., 1999) and another group has published a synthesis of PNA-peptide conjugates (Goodwin et al. 1998). These conjugates provide a carrier for membrane passage that is applicable to the SarA function inhibitors of the present invention. Furthermore, a world leading PNA research group has recently published a PNA conjugate that is readily taken up by cells (Ljungstrom et al., 1999). Further, another group found that polyarginine (9-mer) was very useful as a "cellular pass" and increases the rate of uptake of many drugs significantly (Service, 2000). Peptides are the simplest conjugates to prepare because PNAs are synthesized by the same chemistry methods used to synthesize peptides. All of these efforts are aimed at human diseases and PNA uptake by human cells and applicable in treating Staphylococcus infections. Because the Staphylococci are taking up peptides and oligonucleotides as their colony is growing, coupling PNA and other inhibitors to peptides enhances the uptake of PNA by the bacteria.

Detailed Description Text (104):
In the present invention, PNA and HP molecules are synthesized that are designed to interrupt SarA-agr interactions. Appropriate molecules are selected by in vitro tests first and then as inhibitors of exoprotein expression. Molecules that are found to be effective in these tests are selected for testing in animal models of staphylococcal disease.

Detailed Description Text (112):
Antisense molecules (oligonucleotide analogs) to the sar gene or to the SarA binding sites on the agr locus are also inhibitors of the SarA function involved in the expression of virulence factors in staphylococcal infections. Most commonly, these inhibitors are relatively small RNA or DNA molecules because they can be designed to be highly specific. In general, so-called "antisense" molecules have a sequence which is complementary to a portion of the mRNA.

Detailed Description Text (120):
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Other Reference Publication (1):
Chien et al, "Molecular interactions between two global regulators, sar and agr, in Staphlococcus aureus", J. Biol. Chem. (1998) 273(5):2645-2652.*

Other Reference Publication (2):
Novick et al, "The agr P2 operon: an autocatalytic sensory transduction system in

Staphlococcus aureus", Mol. Gen. Genetics (1995) 248:446-458.*

Other Reference Publication (3):
A. Abdelnour et al., "The Accessory Gene Regulator (agr) Controls . . . Murine Arthritis Model", Infection and Immunity, vol. 61, No. 9, pp. 3879-3885, Sep. 1993, Am. Soc. for Microbiology.

Other Reference Publication (4):
N. Balaban et al., "Autoinducer of Virulence As a Target for Vaccine and Therapy Against Staphylococcus aureus", vol. 280, pp. 438-440, Apr. 17, 1998.

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M. C. Booth et al. "Accessory Gene Regulator Controls Staphylococcus aureus Virulence in Endophthalmitis", Investigative Ophthal. & Visual Sci., vol. 36, No. 9, Aug. 1995, Assoc. for Research in Vision and Ophthal.

Other Reference Publication (6):
A.L. Cheung et al., "Role of the sar Locus of Staphylococcus aureus in Induction of Endocarditis in Rabbits", Infection and Immunity, vol. 62, No. 5, pp. 1719-1725, May 1994, Am. Society for Microbiology.

Other Reference Publication (8):
A.L. Cheung et al., "sar Genetic Determinants Necessary for Transcription of RNAII and RNAIII in the agr Locus of Staphylococcus aureus", J. Bacteriology, pp. 3963-3971, Jun. 1997, (XP-002155701).

Other Reference Publication (9):
Y.T. Chien et al., "Molecular Interactions between Two Global Regulators, sar and agr, in Staphylococcus Aureus", J. Biol. Chem., vol. 273:5, pp. 2645-2652, Jan. 1998, (XP-002155700),.

Other Reference Publication (11):
A.F. Gillaspy et al., "Role of accessory Gene Regulator (agr) in Pathogenesis of Staphylococcal Osteomyelitis" Infection and Immunity, vol. 63, No. 9, pp. 3373-3380, Sep. 1995, Am. Soc. of Microbiology.

Other Reference Publication (15):
E. Morfeldt et al., "Transcriptional control of a agr-dependent virulence gene . . . in Staphylococcus aureus", Molecular Microbiology, vol. 21, No. 6, pp. 1227-1237, 1996, Blackwell Science Ltd.

Other Reference Publication (16):
T.M. Rechtin et al., "Characterization of the SarA virulence gene regulator of Staphylococcus aureus", Mol. Microbiology, vol. 33:2, pp. 307-316, 1999, Blackwell Science Ltd.

CLAIMS:

1. A method of identifying inhibitors of the binding of SarA involved in the expression of staphylococcal virulence genes to at least one SarA binding site comprising: a) contacting a candidate inhibitor with at least one SarA binding site of the agr locus in solution to allow the binding reaction to equilibrate for a sufficient period of time, wherein said SarA binding site of the agr locus as depicted in FIG. 3 is at least one nucleotide sequence selected from the group consisting of at least the nucleotide sequences in the A1 (SEQ ID NO:12) and A2 (SEQ ID NO:14) boxes, at least the nucleotide sequences in the B1 (SEQ ID NO:16) and B2 (SEQ ID NO:17) boxes and at least the nucleotide sequences in the C1 (SEQ ID NO:15) and C2 (SEQ ID NO:13) boxes; b) assessing the binding of said candidate inhibitor to said SarA binding site of the agr locus; and c) selecting said candidate inhibitor that binds to the agr locus.

2. The method of claim 1, wherein said assessment of binding of the inhibitor to said SarA binding site is performed by an electrophoretic mobility shift assay for fluorescence anisotropy.

3. The method of claim 1, wherein said candidate inhibitor interferes with SarA mediated activation of the agr locus.

4. The method of claim 3, wherein said candidate inhibitor interferes with the binding of SarA to at least a portion of the agr locus.

7. The method of claim 6, wherein said candidate inhibitor interferes with the binding of SarA to at least a portion of the agr locus depicted in FIG. 3 (SEQ ID NOS:10 and 11).

8. The method of claim 1, wherein said candidate inhibitor also interferes with the binding of SarA to the intervening nucleotide sequences between the A1 (SEQ ID NO:12) and A2 (SEQ ID NO:14) boxes when the inhibitor binds to the nucleotide sequence in the A1 (SEQ ID NO:12) and A2 (SEQ ID NO:14) boxes, to the intervening nucleotide sequences between the B1 (SEQ ID NO:16) and B2 (SEQ ID NO:17) boxes when the inhibitor binds to the nucleotide sequences in the B1 (SEQ ID NO:16) and B2 (SEQ ID NO:17) boxes or to the intervening nucleotide sequences between the C1 (SEQ ID NO:15) and C2 (SEQ ID NO:13) boxes when the inhibitor binds to the nucleotide sequences in the C1 (SEQ ID NO:15) and C2 (SEQ ID NO:13) boxes.

10. The method of claim 1, wherein said candidate inhibitor is selected from the group consisting of an oligonucleotide analog of the Sar A binding site of the agr locus and a hairpin polyamide.

12. The method of claim 1, further comprising the addition of SarA to the solution of step a) simultaneously with said contacting of said inhibitor and said SarA binding site, and assessing the binding affinity of said candidate inhibitor relative to the binding affinity of said SarA to said SarA binding site of the agr locus.

13. The method of claim 1, further comprising the addition of SarA to the solution of step a) sequentially after said contacting of said inhibitor and said SarA binding site, and assessing the binding affinity of said candidate inhibitor relative to the binding affinity of said SarA to said SarA binding site of the agr locus.

14. The method of claim 10, further comprising the addition of SarA to the solution of step a) simultaneously with said contacting of said inhibitor and said SarA binding site, and assessing the binding affinity of said candidate inhibitor relative to the binding affinity of said SarA to said SarA binding site of the agr locus.

15. The method of claim 10, further comprising the addition of SarA to the solution of step a) sequentially after said contacting of said inhibitor and said SarA binding site, and assessing the binding affinity of said candidate inhibitor relative to the binding affinity of said SarA to said SarA binding site of the agr locus.

16. The method of claim 11, further comprising the addition of SarA to the solution of step a) simultaneously with said contacting of said inhibitor and said SarA binding site, and assessing the binding affinity of said candidate inhibitor relative to the binding affinity of said SarA to said SarA binding site of the agr locus.

17. The method of claim 11, further comprising the addition of SarA to the solution

of step a) sequentially after said contacting of said inhibitor and said SarA binding site, and assessing the binding affinity of said candidate inhibitor relative to the binding affinity of said SarA to said SarA binding site of the agr locus.

18. A method of identifying inhibitors of the binding of SarA involved in the expression of staphylococcal virulence genes to at least one SarA binding site comprising: a) contacting a candidate inhibitor that binds to at least a portion of the agr locus with at least one SarA binding site of the agr locus in solution to allow the binding reaction to equilibrate for a sufficient period of time, wherein said SarA binding site of the agr locus as depicted in FIG. 3 is at least one nucleotide sequence selected from the group consisting of at least the nucleotide sequences in the A1 (SEQ ID NO:12) and A2 (SEQ ID NO:14) boxes, at least the nucleotide sequences in the B1 (SEQ ID NO:16) and B2 (SEQ ID NO:17) boxes and at least the nucleotide sequences in the C1 (SEQ ID NO:15) and C2 (SEQ ID NO:13) boxes; b) assessing the binding of said candidate inhibitor to said SarA binding site of the agr locus by adding SarA to the solution of step a) simultaneously with or sequentially with said contacting of said inhibitor and said SarA binding site, and assessing the binding affinity of said candidate inhibitor relative to the binding affinity of said SarA to said SarA binding site of the agr locus; and c) selecting said candidate inhibitor that binds to the agr locus as assessed relative to the binding affinity of SarA to the agr locus.

19. The method of claim 18, wherein said candidate inhibitor is selected from the group consisting of an oligonucleotide analog of the Sar A binding site of the agr locus and a hairpin polyamide.

21. The method of claim 18, wherein said assessment of binding of the inhibitor to said SarA binding site is performed by an electrophoretic mobility shift assay or fluorescence anisotropy.

24. The method of claim 18, wherein said candidate inhibitor interferes with the binding of SarA to at least a portion of the agr locus depicted in FIG. 3 (SEQ ID NOS:10 and 11).

25. The method of claim 18, wherein said candidate inhibitor also interferes with the binding of SarA to the intervening nucleotide sequences between the A1 (SEQ ID NO:12) and A2 (SEQ ID NO:14) boxes when the inhibitor binds to the nucleotide sequence in the A1 (SEQ ID NO:12) and A2 (SEQ ID NO:14) boxes, to the intervening nucleotide sequences between the B1 (SEQ ID NO:16) and B2 (SEQ ID NO:17) boxes when the inhibitor binds to the nucleotide sequences in the B1 (SEQ ID NO:16) and B2 (SEQ ID NO:17) boxes or to the intervening nucleotide sequences between the C1 (SEQ ID NO:15) and C2 (SEQ ID NO:13) boxes when the inhibitor binds to the nucleotide sequences in the C1 (SEQ ID NO:15) and C2 (SEQ ID NO:13) boxes.

First Hit Fwd Refs

L7: Entry 6 of 11 File: USPT Feb 1, 2000

US-PAT-NO: 6020121
DOCUMENT-IDENTIFIER: US 6020121 A

TITLE: Inhibitors of regulatory pathways

DATE-ISSUED: February 1, 2000

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NAME	CITY	STATE	ZIP CODE	COUNTRY
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Boggs; Amy	Menlo Park	CA		
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Federspiel; Nancy A.	Menlo Park	CA		
Hebert; Alan	Menlo Park	CA		
Hecker; Scott	Los Gatos	CA		
Malouin; Francois	Los Gatos	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Microcide Pharmaceuticals, Inc.	Mountain View	CA			02

APPL-NO: 08/672215 [PALM]
DATE FILED: June 25, 1996

PARENT-CASE:
RELATED APPLICATIONS The present application claims priority to U.S. Provisional Application Ser. No. 60/004,626, filed Sep. 29, 1995 (Bao et al., INHIBITORS OF REGULATORY PATHWAYS), which is incorporated herein by reference, including any drawings.

INT-CL-ISSUED: [06] C12 Q 1/00, C12 Q 1/68, C12 N 1/38, C12 N 15/31

US-CL-ISSUED: 435/4; 435/6, 435/252.1, 536/23.7, 536/24.1
US-CL-CURRENT: 435/4; 435/252.1, 435/6, 536/23.7, 536/24.1

FIELD-OF-CLASSIFICATION-SEARCH: 514/340, 514/2, 435/6, 435/4, 435/252.1, 536/23.7, 536/24.1
See application file for complete search history.

PRIOR-ART-DISCLOSED:

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	CLASS
9426262	November 1994	WO	

OTHER PUBLICATIONS

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ART-UNIT: 166

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ABSTRACT:

Methods are provided for screening for potential inhibitors of bacterial, or other microbial, global pathogenesis gene regulators and other gene regulators. Methods are also provided for treating microbial (e.g., bacterial) infections using such inhibitors. Also included are pharmaceutical compositions containing such inhibitors. The screening methods involve detecting whether the activity of a global pathogenesis gene regulator is altered in the presence of a test compound.

15 Claims, 20 Drawing figures

US-PAT-NO: 6020121
DOCUMENT-IDENTIFIER: US 6020121 A

TITLE: Inhibitors of regulatory pathways

DATE-ISSUED: February 1, 2000

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/4; 435/252.1, 435/6, 536/23.7, 536/24.1

CLAIMS:

What we claim is:

1. A method of screening for an inhibitor of a global regulator of pathogenesis genes, comprising determining if any of a plurality of different test compounds alters the level of activity of said global regulator.

2. The method of claim 1, comprising determining if said test compound alters the level of expression of said global regulator.

3. The method of claim 2, comprising the steps of:

a) detecting the transcriptional or translational product of a hybrid DNA construct comprising a regulatory region of a gene encoding a global regulator of pathogenesis genes transcriptionally linked with a reporter gene, and

b) determining whether the amount of the transcriptional or translational product of said reporter gene differs in the presence and absence of any of said plurality of different test compounds,

wherein said hybrid DNA construct is incorporated into a bacterium.

4. The method of claim 3, wherein said regulatory region of a gene encoding a global regulator of pathogenesis genes comprises a P3 promoter.

5. The method of claim 2, wherein said global regulator of pathogenesis genes is from a Staphylococcus strain.

6. The method of claim 5, wherein said Staphylococcal global regulator is from Staphylococcus aureus.

7. The method of claim 5, wherein said global regulator is encoded by the agr locus.

8. The method of claim 5, wherein said global regulator is encoded by the xpr gene.

9. The method of claim 5, wherein said global regulator is encoded by the sar gene.

10. The method of claim 5, wherein said global regulator is encoded by the sae gene.

11. A method of screening for an inhibitor of a Staphylococcal global regulator of pathogenesis genes, comprising contacting a plurality of different test compounds with said Staphylococcal strain, and determining if any of said plurality of different test compounds alters the level of expression of an RNAIII transcript.

12. The method of claim 11 wherein said Staphyloccocal strain is Staphylococcus aureus.

13. The method of claim 11, further comprising the steps of:

a) detecting the transcriptional or translational product of a hybrid DNA construct comprising a regulatory region of a gene encoding RNAIII transcriptionally linked with a reporter gene, and

b) determining whether the amount of the transcriptional or translational product of said reporter gene differs in the presence and absence of any of said plurality of different test compounds,

wherein said hybrid DNA construct is incorporated into a bacterium.

14. The method of claim 13, wherein said regulatory region comprises a P3 promoter.

15. The method of either of claims 11 or 12, wherein the level of RNAIII expression is reduced.

First Hit Fwd Refs

L7: Entry 7 of 11 File: USPT Nov 2, 1999

US-PAT-NO: 5976792
DOCUMENT-IDENTIFIER: US 5976792 A

TITLE: Regulation of exoprotein in staphylococcus aureus

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/6; 435/320.1, 530/350, 530/387.1, 530/388.1, 530/388.4, 530/825, 536/23.7, 536/24.32

CLAIMS:

What is claimed is:

1. An isolated, purified full-length S. aureus staphylococcal accessory regulatory (sar) protein, wherein said protein regulates the expression of S. aureus exoprotein virulence determinants, and has an amino acid sequence of about 124 amino acids.

2. A sar protein according to claim 1, wherein the protein comprises the amino acid sequence of the wild-type sar protein of SEQ ID NO: 7.

3. A fragment of a sar protein according to claim 1, wherein said fragment is selected from the group consisting of the sarA protein, the ORF3 protein, and the sart protein.

4. A fragment of a sar protein according to claim 3, wherein said fragment consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 12 and SEQ ID NO: 13.

5. An isolated, purified DNA molecule from S. aureus encoding a sar protein according to claim 1, wherein said DNA molecule consists of nucleotide sequences of the sar regulatory region.

6. A DNA molecule according to claim 5, wherein said DNA molecule encodes a sar protein which comprises the amino acid sequence of SEQ ID NO: 7.

7. A DNA molecule according to claim 5, wherein said DNA molecule encodes a sar protein which comprises an amino acid sequence which is degenerate to the sequence of SEQ ID NO: 7.

8. A DNA molecule according to claim 5, wherein said DNA molecule has a conservative mutation.

9. A replicable expression vector comprising a DNA molecule according to claim 5.

10. A replicable expression vector according to claim 9, wherein said DNA molecule is operably linked to elements required for its expression.

11. A replicable expression vector according to claim 10, wherein the nucleotide sequence element comprises a nucleotide sequence selected from the group consisting of a promoter, a transcription enhancer element, a termination signal, a translation signal, and a combination of two or more of these elements including a promoter.

12. A replicable expression vector according to claim 11, wherein the promoter is selected from the group consisting of trp, lac, P.sub.L, and T7 polymerase.

13. A replicable expression vector according to claim 10, further comprising a selectable marker.

14. A replicable expression vector according to claim 10, wherein the vector is selected from the group consisting of plasmids, bacteriophages, cosmids, and viruses.

15. A replicable expression vector according to claim 10, wherein the expression vector comprises RNA.

16. An isolated, purified antibody to the sar protein of claim 1.

17. A composition comprising the antibody of claim 16 linked to a reporter molecule.

18. The composition of claim 17, wherein the reporter molecule is selected from the group consisting of enzymes, fluorophores, and radionuclide containing molecules.

19. A diagnostic method for detecting the presence of the sar gene in a microbial isolate, comprising

extracting the DNA of the microbial isolate, and

probing said DNA with a labeled gene probe constructed from a DNA molecule according to claim 5, wherein detection of hybridization is indicative of the presence of the sar gene in said microbial isolate.

20. An isolated, purified S. aureus staphylococcal accessory regulatory (sarA) protein, wherein said protein has a molecular weight from about 14.7 to about 14.8 kD, a pI of about 8.5, and an ammo acid sequence selected from the group consisting of SEQ ID NO: 7; SEQ ID NO: 12; and SEQ ID NO: 13.

21. A fusion protein comprising a sarA protein according to claim 20 linked to a heterologous protein.

22. An isolated, purified DNA molecule encoding a sarA protein according to claim 20.

23. A replicable expression vector comprising a DNA molecule according to claim 22.

24. A replicable expression vector according to claim 23, wherein said DNA molecule is operably linked to elements required for its expression.

25. A replicable expression vector according to claim 24, wherein said elements are selected from the group consisting of a promoter, a transcription enhancer element, a termination signal, a translation signal, and a combination of two or more of these elements including a promoter.

26. A replicable expression vector according to claim 25, wherein the promoter is selected from the group consisting of trp, lac, P.sub.L, and T7 polymerase.

27. A replicable expression vector according to claim 26, further comprising a selectable marker.

28. The replicable expression vector of claim 23, wherein the vector is selected from the group consisting of plasmids, bacteriophages, cosmids, and viruses.

29. The replicable expression vector of claim 23, wherein the expression vector comprises RNA.

30. A diagnostic method for detecting the presence of the sar gene in a microbial isolate, comprising

extracting the DNA of the microbial isolate, and

probing said DNA with a labeled gene probe constructed from the DNA of claim 24, wherein detection of hybridization is indicative of the presence of the sar gene in said microbial isolate.

US-PAT-NO: 5587288
DOCUMENT-IDENTIFIER: US 5587288 A
** See image for Certificate of Correction **

TITLE: Regulation of exoprotein in Staphylococcus aureus

DATE-ISSUED: December 24, 1996

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/6; 530/350, 536/23.1, 536/24.3

CLAIMS:

What is claimed is:

1. An isolated, purified S. aureus staphylococcal accessory regulatory (sar) protein, consisting of the amino acid sequence shown in SEQ ID NO. 1.

2. An isolated, purified S. aureus gene sar comprising a contiguous nucleotide sequence encoding the sar protein of claim 1.

3. A sar gene according to claim 1, wherein the gone consists of the nucleotide sequence of SEQ ID NO. 1.